FORM PTO-1390 (REV 11-2000)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 3557-12
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) 10/088025 Unknown
INTERNATIONAL APPLICATION NO. **PCT/EP00/07625**	INTERNATIONAL FILING DATE **5 August 2000**	PRIORITY DATE CLAIMED **15 September 1999**

TITLE OF INVENTION
PLANTS WITH A MODIFIED AMINO ACID CONTENT AND THEIR GENERATION

APPLICANT(S) FOR DO/EO/US
REINDL et al

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The U.S. has been elected by the expiration of 19 months from the priority date (Article 31).
5. A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has **NOT** expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 To 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information. PTO-1449 and copy of International Search Report

U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/088025 Unknown	PCT/EP00/07625	3557-12

21. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 C.F.R. 1.492(a)(1)-(5):		
-- Neither international preliminary examination fee (37 C.F.R. 1.482) nor international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1040.00		
-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $890.00		
-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO $740.00		
-- International preliminary examination fee (37 C.F.R. 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) $710.00		
-- International preliminary examination fee (37 C.F.R. 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 890.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. 1.492(e)).	$ 0.00	

CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE			
Total Claims	19	-20 =	0	X	$18.00	$ 0.00	
Independent Claims	1	-3 =	0	X	$84.00	0.00	
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)					$280.00	$ 280.00	
TOTAL OF ABOVE CALCULATIONS =						$ **1170.00**	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.						0.00	
SUBTOTAL =						$ **1170.00**	
Processing fee of $130.00, for furnishing the English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. 1.492(f)). +						0.00	
TOTAL NATIONAL FEE =						$ **1170.00**	
Fee for recording the enclosed assignment (37 C.F.R. 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. 3.28, 3.31). **$40.00** per property +						$ 0.00	
Fee for Petition to Revive Unintentionally Abandoned Application ($1280.00 – Small Entity = $640.00)						$ 0.00	
TOTAL FEES ENCLOSED =						$ **1170.00**	
						Amount to be: refunded	$
						Charged	$

a. ☒ A check in the amount of $1170.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 14-1140 in the amount of $_____ to cover the above fees. A duplicate copy of this form is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-1140. A duplicate copy of this form is enclosed.
d. ☒ The entire content of the foreign application(s), referred to in this application is/are hereby incorporated by reference in this application.

NOTE: Where an appropriate time limit under 37 C.F.R. 1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

NIXON & VANDERHYE P.C.
1100 North Glebe Road, 8th Floor
Arlington, Virginia 22201-4714
Telephone: (703) 816-4000

SIGNATURE

B. J. Sadoff
NAME

36,663 **March 14, 2002**
REGISTRATION NUMBER Date

#57

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

REINDL et al

Atty. Ref.: 3557-12

Serial No. 10/088,025

Group:

Filed: March 14, 2002

Examiner:

For: PLANTS WITH A MODIFIED AMINO ACID CONTENT AND THEIR GENERATION

* * * * * * * * * * *

September 13, 2002

Honorable Assistant Commissioner of Patents
Washington, DC 20231

Sir:

AMENDMENT

Responsive to the Notification dated May 15, 2002, entry and consideration of the following amendments and remarks are requested; the period for response having been extended up to and including September 15, 2002, by submission of the requisite petition and fee, attached.

IN THE SPECIFICATION:

Amend the specification as follows.

Delete the paragraph spanning page 5, lines 29-33 and insert the following therefor:

--The invention furthermore relates to an ATP/ADP translocator gene for use in one of the above-described plants with an Arabidopsis thaliana amino acid sequence (EMBL Accession No. Z49227) encoding by the nucleotide sequence shown in Fig. 1 (SEQ ID NO:1).--

In re Application of: REINDL et al
Serial No. 10/088,025

Insert the attached revised Figures 1 and 2 in place of the similarly-numbered, originally-filed Figures.

Please insert the attached Sequence Listing after the claims pages.

IN THE CLAIMS:

Amend the claims as follows:

6. (Twice Amended) ATP/ADP translocator gene for use in a plant according to Claim 1 with an Arabidopsis thaliana amino acid sequence (EMBL Accession No. Z49227) encoded by the nucleotide sequence shown in Fig. 1 (SEQ ID NO:1).

REMARKS

Reconsideration is requested.

Responsive to the Notification dated May 15, 2002, a copy of the requisite Declaration is attached.

A copy of the Notification dated May 15, 2002 is attached.

The specification has been amended to include the attached revised Figures. A marked-up copy of the amended Figures is attached wherein changes are shown as underlined text.

The specification has been amended to include the attached Sequence Listing. The specification has been amended to include sequence identifiers, consistent with the attached Sequence Listing. The attached paper and computer readable copies of the Sequence Listing are the same. No new matter has been added. A separate Statement to this effect is attached.

An early and favorable Action on the merits is requested.

In re Application of: REINDL et al
Serial No. 10/088,025

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____

B. J. Sadoff
Reg. No. 36,663

1100 North Glebe Road, 8th Floor
Arlington, VA 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

In re Application of: REINDL et al
Serial No. 10/088,025

MARKED-UP COPY OF AMENDED SPECIFICAITON AND CLAIMS

IN THE SPECIFICATION:

Amend the specification as follows.

Delete the paragraph spanning page 5, lines 29-33 and insert the following therefor:

--The invention furthermore relates to an ATP/ADP translocator gene for use in one of the above-described plants with an Arabidopsis thaliana [nucleotide] amino acid sequence (EMBL Accession No. Z49227) encoding [the amino acid] by the nucleotide sequence shown in Fig. 1 (SEQ ID NO:1).--

IN THE CLAIMS:

Amend the claims as follows:

6. (Twice Amended) ATP/ADP translocator gene for use in a plant according to Claim 1 with an Arabidopsis thaliana [nucleotide sequence] amino acid sequence (EMBL Accession No. Z49227) [encoding the amino acid] encoded by the nucleotide sequence shown in Fig. 1 (SEQ ID NO:1).

Fig. 1: Arabidopsis thaliana cDNA corresponding to the coding region of the chloroplast ATP/ADP translocator 1 (EMBL Accession Number Z49227) (SEQ ID NO:1)

atggaagctgtgattcaaaccagagggcttctctctttacccaccaaacccatcggagtgagaagcca
acttcagccttcccatggcttaaagcagagacttttcgccgcgaagccaagaaatctacatgggtgtct
ctatcctttaacgggcacaagaaatttcaaacctttgagccaaccctgcatgggatttcgatttcccaca
aagagagaagcaccgagttcatatgcaaggcggaggcgcggctgctggcgacggagctgtcttcg
gcgaagcgattccgcagctgttgtagcctcgcggaagattttcggtgtggaggttgcaaccttgaaaaa
gattatccctttaggattgatgttcttttgtattcttttcaattacacaattctgagggatacaaaggatgtcttg
gtggtgacggcgaaaggaagttctgctgagattatacctttcttgaagacttgggtgaatcttcctatggc
cattgggtttatgctcctctacactaaactctccaatgttctctccaagaaggctctgttttacactgttattgtc
cctttcatcatctactttgggggctttggtttcgtcatgtaccctctcagcaactatattcacccggaagctct
cgcagataagctccttacaaccctcggcccaagattcatgggtcctattgcaatattgcggatttggagtt
tctgtttgttttatgttatggctgagctttggggtagtgtggtggtctcagttctcttctggggctttgctaatcag
atcacaactgtggatgaagccaagaaattctatcctttgttcggcattggagccaatgttgcactgattttc
tcaggaagaaccgtgaaatacttctctaacttgagaaagaatcttggtcctggagttgacggcagtttcg
ttgaaagccatgatgagcattgtggtgggaatgggactcgcatttgtctctctattggtgggtcgaataga
tatgttcctcttccaacccgtagcaagaacaagaaggagaaaccgaagatgggaacgatggaaag
cttgaagttcttggtatcatcaccatacattagagatcttgctactttagtggtggcatacggtattagtatca
atcttgtggaagtcacatggaaatcaaagcttaaagctcagttccctagcccgaatgagtactcagcatt
tatgggagcattctcaacctgcacgggtgttgcaacattcacaatgatgcttctcagccaatacgtattca
ataagtatggttggggagtagctgcaaagatcaccccaactgttctgctattgactggtgttgcgttcttct
ctctaatattgtttggcggcccattcgcaccacttgttgccaagcttggtatgacaccgctacttgcagctgt
gtatgtcggtgcccttcagaatatcttcagcaagagtgccaagtacagcttgttcgacccttgcaaagaa
atggcctatatcccattggatgaggacaccaaggttaaaggcaaagctgcgattgacgtggtctgcaa
cccattaggaaaatcaggggagctttaatacagcagttcatgatcttatcctttggatcactagcgaatt
caacgccgtatctaggaatgatcttgttggttattgtcactgcgtggttagctgcagctaagtcgctggag
ggacagttcaacagcttgcgtctgaagaagagcttgagaaggaaatggagagagcttcatcggtga

Fig. 2: Solanum tuberosum cDNA corresponding to the coding region of the chloroplast ATP/ADP translocator 1 (EMBL Accession Number Y10821) (SEQ ID NO:2)

atggaaggtgttttacaaacaagagggcttctttctttgccttctaaacccaaaatcaaggctttttacccat
tgcctcaaggggtctaaggaacagattcaattctttaagtagtttaaagcctaatcctcttaatggggttt
ctttatcttcaaatgggtttcaaaaagttcaaggctttgacacaaagcctcagttgtttggccaaaagaag
aggtgttttccaatatgcaaagctgaggctgctgctgctggtgcagctgatggacagccacttttgtt
gaaaaggagcaacctaagtttatggggattgaacttgtgacccttaagaaaattataccacttggggcg
atgttcttttgtattctgtttaattatacaatccttagggatactaaggatgtgttggttgtaacagctaaaggg
tccagtgctgagattatccctttcttgaaaacttgggtgaatttgcctatggctattggattcatgcttttgtac
acaaagttggctaatgtgttgtcaaaggaggctctttttatactgttatacttccttttattgcattctttggggc
gtttggttttgttttgtatcctcttagcaattactttcaccctacagcttttgctgataagcttctcaataccttgg
tccaagatttcttggaccaattgctattctgaggatctggagtttctgcttgttctatgtcatggctgagctttg
gggaagtgtggtggtttcagtactcttttggggatttgctaatcagatcacgactgtcgatgaggctaaga
gattctatcctttgtttggacttggagcgaatgttgctcttattttctctggtcgcacagtgaagtacttttctag
cttgagaagctctttaggtcctggagttgatggttgggctatctccctgaaaggaatgatgagtattgttgt
gatgatgggtggggcaatctgtttcttttactggtgggtgaatagaaatgttgctctcccaactcgtagcaa
gaagaagaaggtaaaacctaacatgaccacaatggagagcttgaagttcttggtctcttcaaaatatat
cagggatcttgccacattggttgtagcatatggcattagtatcaaccttgttgaagttacatggaagtcaa
agctcaaagctcagttcccaagccccaatgaatactcctcattcatgggtgacttctcaactgctactgg
aatagcaactttcacaatgatgttgttaagtcaatggattttcgacaagtatgggtggggagcagcagcc
aagataacacctacagtcttgctccttaccggagttggtttcttctccctgcttttgtttggggcacctctagc
acctactcttgcgaagtttggaatgactcctcttctagcagctgtctatgtgggtgcaatgcagaacattttc
agtaagagtgcaaagtatagtttgtttgacccctgcaaagaaatggcctacattcctttggatgaggaca
ccaaggttaaagggaaggcagcaatcgatgttgtctgcaatccactgggaaagtctggaggagctttg
atacaacagttcatgattttgacttttggttcacttgccagctcgacaccctaccttggcggtgtgctcttagt
aattgttcttgcatggttgggagcagccaagtctttggatggacagttcactcaattacgccaagaagaa
gatcttgagaaggaaatggagagagcatcgttgaagatccctgtcgtgtctcaaaatgaaaatggaa
atggtcctctctcaagtgagtcatcactaaatcccgctggaggtgactctaccaacgcttcatcggaacc
ctcctccccaaggagcctgtaa

Marked-Up Copy of Amended Figures

Fig. 1: Arabidopsis thaliana cDNA corresponding to the coding region of the chloroplast ATP/ADP translocator 1 (EMBL Accession Number Z49227) (SEQ ID NO:1)

atggaagctgtgattcaaaccagagggcttctctctttacccaccaaacccatcggagtgagaagcca
acttcagccttcccatggcttaaagcagagacttttcgccgcgaagccaagaaatctacatgggtgtct
ctatcctttaacgggcacaagaaatttcaaacctttgagccaaccctgcatgggatttcgatttcccaca
aagagagaagcaccgagttcatatgcaaggcggaggcgcggctgctggcgacggagctgtcttcg
gcgaagcgattccgcagctgttgtagcctcgcggaagattttcggtgtggaggttgcaaccttgaaaaa
gattatccctttaggattgatgttcttttgtattcttttcaattacacaattctgagggatacaaaggatgtcttg
gtggtgacggcgaaaggaagttctgctgagattatacctttcttgaagacttgggtgaatcttcctatggc
cattgggtttatgctcctctacactaaactctccaatgttctctccaagaaggctctgttttacactgttattgtc
cctttcatcatctactttgggggctttggtttcgtcatgtaccctctcagcaactatattcacccggaagctct
cgcagataagctccttacaaccctcggcccaagattcatgggtcctattgcaatattgcggatttggagtt
tctgtttgttttatgttatggctgagctttggggtagtgtggtggtctcagttctcttctggggctttgctaatcag
atcacaactgtggatgaagccaagaaattctatcctttgttcggcattggagccaatgttgcactgattttc
tcaggaagaaccgtgaaatacttctctaacttgagaaagaatcttggtcctggagttgacggcagtttcg
ttgaaagccatgatgagcattgtggtgggaatgggactcgcatttgtctctctattggtgggtcgaataga
tatgttcctcttccaacccgtagcaagaacaagaaggagaaaccgaagatgggaacgatggaaag
cttgaagttcttggtatcatcaccatacattagagatcttgctactttagtggtggcatacggtattagtatca
atcttgtggaagtcacatggaaatcaaagcttaaagctcagttccctagcccgaatgagtactcagcatt
tatgggagcattctcaacctgcacgggtgttgcaacattcacaatgatgcttctcagccaatacgtattca
ataagtatggttggggagtagctgcaaagatcaccccaactgttctgctattgactggtgttgcgttcttct
ctctaatattgtttggcggcccattcgcaccacttgttgccaagcttggtatgacaccgctacttgcagctgt
gtatgtcggtgcccttcagaatatcttcagcaagagtgccaagtacagcttgttcgacccttgcaaagaa
atggcctatatcccattggatgaggacaccaaggttaaaggcaaagctgcgattgacgtggtctgcaa
cccattaggaaaatcaggggagctttaatacagcagttcatgatcttatcctttggatcactagcgaatt
caacgccgtatctaggaatgatcttgttggttattgtcactgcgtggttagctgcagctaagtcgctggag
ggacagttcaacagcttgcgtctgaagaagagcttgagaaggaaatggagagagcttcatcggtga

Marked-Up Copy of Amended Figures

Fig. 2: Solanum tuberosum cDNA corresponding to the coding region of the chloroplast ATP/ADP translocator 1 (EMBL Accession Number Y10821) (SEQ ID NO:2)

atggaaggtgttttacaaacaagagggcttctttctttgccttctaaacccaaaatcaaggctttttacccat
tgcctcaaggggtctaaggaacagattcaattctttaagtagtttaaagcctaatcctcttaatggggttt
ctttatcttcaaatgggtttcaaaaagttcaaggctttgacacaaagcctcagttgtttggccaaaagaag
aggtgttttccaatatgcaaagctgaggctgctgctgctgctggtgcagctgatggacagccacttttttgtt
gaaaaggagcaacctaagtttatggggattgaacttgtgacccttaagaaaattataccacttggggcg
atgttcttttgtattctgtttaattatacaatccttagggatactaaggatgtgttggttgtaacagctaaaggg
tccagtgctgagattatccctttcttgaaaacttgggtgaatttgcctatggctattggattcatgcttttgtac
acaaagttggctaatgtgttgtcaaaggaggctcttttttatactgttatacttccttttattgcattctttggggc
gtttggttttgttttgtatcctcttagcaattactttcaccctacagcttttgctgataagcttctcaataccttgg
tccaagatttcttggaccaattgctattctgaggatctggagtttctgcttgttctatgtcatggctgagctttg
gggaagtgtggtggtttcagtactcttttggggatttgctaatcagatcacgactgtcgatgaggctaaga
gattctatcctttgtttggacttggagcgaatgttgctcttattttctctggtcgcacagtgaagtacttttctag
cttgagaagctctttaggtcctggagttgatggttgggctatctccctgaaaggaatgatgagtattgttgt
gatgatgggtggggcaatctgtttcttttactggtgggtgaatagaaatgttgctctcccaactcgtagcaa
gaagaagaaggtaaaacctaacatgaccacaatggagagcttgaagttcttggtctcttcaaaatatat
cagggatcttgccacattggttgtagcatatggcattagtatcaaccttgttgaagttacatggaagtcaa
agctcaaagctcagttcccaagccccaatgaatactcctcattcatgggtgacttctcaactgctactgg
aatagcaactttcacaatgatgttgttaagtcaatggattttcgacaagtatgggtggggagcagcagcc
aagataacacctacagtcttgctccttaccggagttggtttcttctccctgcttttgtttggggcacctctagc
acctactcttgcgaagtttggaatgactcctcttctagcagctgtctatgtgggtgcaatgcagaacattttc
agtaagagtgcaaagtatagtttgtttgacccctgcaaagaaatggcctacattcctttggatgaggaca
ccaaggttaaagggaaggcagcaatcgatgttgtctgcaatccactgggaaagtctggaggagctttg
atacaacagttcatgattttgacttttggttcacttgccagctcgacaccctaccttggcggtgtgctcttagt
aattgttcttgcatggttgggagcagccaagtctttggatggacagttcactcaattacgccaagaagaa
gatcttgagaaggaaatggagagagcatcgttgaagatccctgtcgtgtctcaaaatgaaaatggaa
atggtcctctctcaagtgagtcatcactaaatcccgctggaggtgactctaccaacgcttcatcggaacc
ctcctccccaaggagcctgtaa

REINDL et al

Atty. Ref.: 3557-12

Serial No. 10/088,025

Group:

Filed: March 14, 2002

Examiner:

For: PLANTS WITH A MODIFIED AMINO ACID CONTENT AND THEIR GENERATION

* * * * * * * * * * * *

September 13, 2002

Honorable Assistant Commissioner of Patents
Washington, DC 20231

Sir:

STATEMENT

The attached paper and computer readable copies of the Sequence Listing are the same. No new matter has been added.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____

B. J. Sadoff
Reg. No. 36,663

1100 North Glebe Road, 8th Floor
Arlington, VA 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

REINDL et al

Atty. Ref.: **3557-12**

Serial No. **Unknown**

Group:

National Phase of: **PCT/EP00/07625**
International Filing Date: **5 August 2000**

Filed: **March 14, 2002**

Examiner:

For: **PLANTS WITH A MODIFIED AMINO ACID CONTENT AND THEIR GENERATION**

* * * * * * * * * * * * *

March 14, 2002

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

Prior to calculation of the filing fee and in order to place the above identified application in better condition for examination, please amend as follows:

IN THE SPECIFICATION

Page 1, after the title insert the following:

-- This application is the US national phase of international application PCT/EP00/07625 filed August 5, 2000 which designated the U.S. --.

IN THE CLAIMS

Please substitute the following amended claims for corresponding claims previously presented. A copy of the amended claims showing current revisions is attached.

4. (Amended) Transformed plant and its progeny according to claim 1, characterized in that it exhibits one or more essential amino acid(s) whose content is increased over that of the untransformed plant.

5. (Amended) Transformed plant and its progeny according to claim 1, characterized in that it is a useful plant.

6. (Amended) ATP/ADP translocator gene for use in a plant according to claim 1 with an Arabidopsis thaliana nucleotide sequence(EMBL Accession No. Z49227) encoding the amino acid sequence shown in Fig. 1.

9. (Amended) ATP/ADP translocator gene according to claim 6 with an upstream, operably linked promoter.

10. (Amended) Gene structure comprising an ATP/ADP translocator gene according to claim 6 and regula-tory sequences linked operably to this gene.

11. (Amended) Vector comprising an ATP/ADP translocator gene according to claim 6.

13. (Amended) Seeds of the plant according to claim 1.

14. Tissue or cells or material capable of propagation from the plant according to claim 1.

15. (Amended) Method of generating a plant with an increased amino acid content, characterized in that an ATP/ADP translocator gene according to claim 6 is transferred by recombinant methods.

16. (Amended) Use of the transformed plant according to claim 1 as useful plant or fodder plant.

17. (Amended) Use of the transformed plants according to claim 1 or of tissue or cells thereof or of extracts thereof in sectors of agriculture, the feedstuff industry, the pharmaceutical industry or in the health sector.

REINDL et al
Serial No. **Unknown**

REMARKS

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**Version with markings to show changes made**."

The above amendments are made to place the claims in a more traditional format.

Respectfully submitted,
NIXON & VANDERHYE P.C.
By: _____
B. J. Sadoff
Reg. No. **36,663**

BJS:lmy

1100 North Glebe Road, 8th Floor
Arlington, VA 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

VERSION WITH MARKINGS TO SHOW CHANGES MADE

4. (Amended) Transformed plant and its progeny according to [one of Claims 1 to 3] claim 1, characterized in that it exhibits one or more essential amino acid(s) whose content is increased over that of the untransformed plant.

5. (Amended) Transformed plant and its progeny according to [one of Claims 1 to 4] claim 1, characterized in that it is a useful plant.

6. (Amended) ATP/ADP translocator gene for use in a plant according to [one of Claims 1 to 5] claim 1 with an Arabidopsis thaliana nucleotide sequence(EMBL Accession No. Z49227) encoding the amino acid sequence shown in Fig. 1.

9. (Amended) ATP/ADP translocator gene according to [one of Claims 6 to 8] claim 6 with an upstream, operably linked promoter.

10. (Amended) Gene structure comprising an ATP/ADP translocator gene according to [one of Claims 6 to 9] claim 6 and regula-tory sequences linked operably to this gene.

11. (Amended) Vector comprising an ATP/ADP translocator gene according to [one of Claims 6 to 9 or a gene structure according to Claim 10] claim 6.

13. (Amended) Seeds of the plant according to [one of Claims 1 to 5] claim 1.

14. Tissue or cells or material capable of propagation from the plant according to [one of Claims 1 to 5] claim 1.

15. (Amended) Method of generating a plant with an increased amino acid content [according to one of Claims 1 to 5], characterized in that an ATP/ADP translocator gene according to [one of Claims 6 to 9 or a gene structure according to

Claim 10 or a vector according to Claim 11 or 12] claim 6 is transferred by recombinant methods.

16. (Amended) Use of the transformed plant according to [one of Claims 1 to 5] claim 1 as useful plant or fodder plant.

17. (Amended) Use of the transformed plants according to [one of Claims 1 to 5] claim 1 or of tissue or cells thereof or of extracts thereof in sectors of agriculture, the feedstuff industry, the pharmaceutical industry or in the health sector.

Plants with a modified amino acid content and their generation

The present invention relates to transformed plants and their progeny whose regulatory sequences and/or gene copy number of the ATP/ADP translocator gene are modified in such a way that they exhibit one or more amino acids simultaneously in modified amounts in comparison with an untransformed plant. The present invention furthermore relates to a method of generating these plants and to their use as useful plant or in sectors of the feedstuff industry.

Humans and animals are only capable of synthesizing 11 out of the 20 amino acids and therefore depend on taking up the 9 what are known as essential amino acids via the food. The nutrition of humans and livestock is predominantly based on plant components. The essential amino acids include lysine, tryptophan, valine, leucine, isoleucine, methionine, threonine, phenylalanine and histidine.

The fact that the concentration of these amino acids in food plants is frequently only very low gives rise to a problem. This is why grain mixtures and vegetable-based foodstuffs are frequently supplemented with synthetically produced amino acids in order to increase their nutritional value.

In the past, a number of avenues were followed to increase the amounts of free amino acids, i.e. amino acids which are not found in proteins. However, these attempts focused mainly on traditional breeding and on the selection of mutants.

In the recent past, there have been increasing attempts to increase the amounts of essential amino acids by applying molecular-genetic techniques. WO 97/28247,

WO 98/13506 and WO 97/35023 describe first attempts at extending the heterologous expression of a seed-specific storage protein which is high in lysine or methionine. The disadvantage here is that the amino acids are stored in proteins, that is to say that, again, the increase takes the form of an increase in bound amino acids.

Furthermore, a large number of attempts for directly controlling amino acid biosynthesis are known. In these attempts, individual genes encoding specific amino acid biosynthesis enzymes were overexpressed in plants, resulting in an increase in the biosynthesis end products in question.

As an alternative, it has furthermore been attempted to control the reaction kinetics of enzymes. What is known as the product inhibition of enzymes causes a particular problem here. For example, Shaul and Galili (1993; Plant Mol Biol 23: 759-768) and Falco et al. (1995; Bio/Technology 13: 577-582) describe plants which overproduce free lysine, coupled with a decrease in free threonine. The enzyme responsible is aspartate kinase, the first enzyme in the biosynthesis of the amino acids derived from aspartate and which is inhibited allosterically by lysine. To circumvent this feedback inhibition, recombinantly modified aspartate kinase genes were overexpressed in plants (WO 94/25605). This modified aspartate kinase has a greatly reduced feedback inhibition by lysine and threonine, leading to an increase in lysine. This aspartate kinase which is insensitive to feedback inhibition by lysine was furthermore overexpressed together with other biosynthesis enzymes. Suitable experiments were carried out in Corynebacteria (1991, Applied and Environmental Microbiology 57: 1746-1752). In these bacteria, however, not only an increase in lysine results, but also a pronounced decrease in the growth rate, which, in turn, has a negative effect on

the lysine balance.

Experiments with plants having both a feedback-insensitive aspartate kinase and a feedback-insensitive dihydropicolinate synthase are described by Shaul and Galili (1993; Plant Mol Biol 23: 759-768). These two enzymes have a key position in amino acid biosynthesis. However, overexpression of these bottleneck enzymes did not result in the hoped-for increase of the two amino acids lysine and threonine. Rather, only the free lysine content was increased, with the free threonine content simultaneously going down drastically.

Beyond the overexpression of one or two amino acid biosynthesis genes, WO 98/56935, EP 0 854 189 and EP 0 485 970 describe multi-gene approaches which aim at influencing the amounts of one or more amino acids simultaneously in one plant. A prerequisite therefor is the genetic modification of a plant with regard to several genes; i.e. it would be necessary to generate a multi-transgenic plant. However, these methods are very complicated. Moreover, such massive interference with hereditary material of the plant increasingly harbours risks of unpredictable side reactions.

It is an object of the present invention to provide transgenic plants and a method of generating them without the abovementioned disadvantages.

We have found that this object is surprisingly achieved in accordance with the invention by providing a transformed plant whose regulatory sequences and/or gene copy number of an ATP/ADP translocator gene are modified in such a way that it exhibits one or more amino acids simultaneously in modified amounts in comparison with a corresponding untransformed plant.

The transformed plants are distinguished in accordance with the invention by exhibiting predominantly one or more essential amino acid(s) in modified amounts.

In particular, the plants according to the invention exhibit one or more essential amino acid(s) whose content is increased over that of the untransformed plants.

The transformed plants are, in accordance with the invention, useful plants, preferably economically relevant plants, such as, for example, potatoes or maize. However, the present invention is not restricted to these genera.

The present invention relates both to the above-mentioned transformed plants, their seeds and progeny and also to tissue, cells or material capable of propagation derived from these transformed plants.

In one embodiment of the present invention in which the gene encoding the ATP/ADP translocator is overexpressed in accordance with the invention in potatoes, an increase in amino acids which are interesting from the nutritional and economical point of view, such as lysine, methionine, threonine, valine, tryptophan, histidine, isoleucine and leucine, is achieved.

In the transformed plant referred to as line 98, the amount of free lysine is increased by 28%; in the transgenic plant referred to as line 62, the increase in the amount of free lysine is 25.75%. Surprisingly, an at least 25% increase in the lysine content is achieved by increasing the ATP/ADP translocator activity in the plants by only 50%. Furthermore, the amount of methionine in line 98 is increased by 11%. In addition to increased amounts of lysine and methionine, increased amounts of the essential amino acids valine (12% in line 98), tryptophan (50% in line 98), threonine (12.5% in line 98), histidine (23.5% in line 98 and 20% in line 62), isoleucine (25% in line 98) and leucine (40% in line 98) are also found.

Accordingly, overexpression of the ATP/ADP translocator in antisense orientation results in a reduction of the amounts of amino acid in the respective transformed plants, referred to as lines 594 and 595. In the case of lysine, only approximately a quarter of the wild-type lysine quantity is found here; while in the case of methionine only approximately not more than one eighth of the wild-type methionine quantity is found.

An overview over the amino acid spectrum in the wild type of potato, Solanum tuberosum, and in transformed potato plants is compiled in Table 1. In this embodiment of the invention, the total amount of free amino acids in the transformed potato plants is increased by approximately 7% over the wild type.

A particular advantage of the present invention is that the increased expression of a single gene, viz. of the ATP/ADP translocator, can bring about a specific increase of several, predominantly essential, amino acids simultaneously.

In accordance with the invention, the transformed plant is distinguished by the fact that it has an increased transport capacity for ATP into the chloroplast membrane.

The invention furthermore relates to an ATP/ADP translocator gene for use in one of the above-described plants with an Arabidopsis thaliana nucleotide sequence (EMBL Accession No. Z49227) encoding the amino acid sequence shown in Fig. 1.

In accordance with the invention, the use of any ATP/ADP translocator gene from organisms which have chloroplasts is feasible. Preferred organisms are plants in general, green algae or mosses.

Normally, the ATP/ADP translocator gene is localized in the internal chloroplast membrane, where it is responsible for the antiport, i.e. the opposite transport, of ATP and ADP, by exporting chloroplast ADP into the cytosol in exchange for ATP. Owing to the increased activity of this ATP/ADP translocator, the amount of ATP in the chloroplast is increased (Neuhaus et al., 1997, The Plant Journal 11: 73-82). Tjaden et al. (1998, Plant Journal 16: 531-540) demonstrated that the uptake of ATP into potato chloroplasts owing to overexpression of the ATP/ADP translocator out performs the uptake capacity of the wild type by an average of 50%. These energy-rich ATP molecules, which are now increasingly available, can be exploited for the increased biosynthesis of starch and fatty acids as described by Möhlmann et al., 1994, Planta, 194: 492-497; Neuhaus et al., 1993, Plant Physiology 101: 573-578; Tjaden et al., 1998, Plant Journal 16: 531-540.

In accordance with the invention, an ATP/ADP translocator gene with a naturally, chemically synthesized, modified, artificially generated nucleotide sequence with essentially the same action or with heterologous nucleotide sequences encoding an ATP/ADP translocator or allelic variations or isoforms thereof or with mixtures thereof may also be employed.

Sequences which encode an ATP/ADP translocator gene with essentially the same action are those sequences which, despite a deviating nucleotide sequence, retain the desired functions. Equivalents with the same action thus encompass naturally occurring variants of the described sequences, but also artificial nucleotide sequences, for example those obtained by chemical synthesis, which are adapted to the codon usage of a plant.

A nucleotide sequence with the same action is also

understood as meaning, in particular, natural or artificial mutations of an originally isolated sequence encoding an ATP/ADP translocator and retaining the desired function. Mutations encompass substitutions, additions, deletions, exchanges or insertions of one or more nucleotide residues. Thus, for example, the present invention also extends to those nucleotide sequences which are obtained by modifying the ATP/ADP translocator nucleotide sequence. The purpose of such a modification may be, for example, the further delimitation of the coding sequence contained therein, or else, for example, the insertion of further cleavage sites for restriction enzymes.

Nucleotide sequences with the same action are also those variants whose function is reduced or increased compared with the original gene or gene fragment.

Suitable DNA sequences are, furthermore, artificial DNA sequences as long as they confer the desired properties, as described above. Such artificial DNA sequences can be determined for example by back-translating proteins constructed by means of molecular modelling which have an ATP/ADP translocator activity, or else by in-vitro selection. Especially suitable are coding DNA sequences which have been obtained by back-translating a polypeptide sequence in accordance with the host-plant-specific codon usage. The specific codon usage can be determined readily by a skilled worker familiar with plant genetic methods by means of computer evaluations of other, known genes of the plant to be transformed.

The invention furthermore encompasses an ATP/ADP translocator gene which is operably linked to regulatory nucleotide sequences. The regulatory sequences also include, inter alia, an upstream promoter which makes possible expression in plants.

Operable linkage is understood as meaning the sequential arrangement of, for example, promoter, coding sequence, terminator and, if appropriate, further regulatory elements in such a way that each of the regulatory elements can fulfil its intended function upon expression of the coding sequence. Suitable as promoter is, in principle, any promoter capable of governing the expression of foreign genes in plants. A plant promoter or a promoter derived from a plant virus is preferably used. Particularly preferred is the cauliflower mosaic virus CaMV 35S promoter (Franck et al., Cell 21 (1980), 285-294). As is known, this promoter contains various recognition sequences for transcriptional effectors which, in their totality, bring about permanent and constitutive expression of the gene introduced (Benfey et al., EMBO J, 8 (1989), 2195-2202).

Further sequences which are preferred for operable linkage, but not limited thereto, are transcription terminators and translation enhancers, such as the tobacco mosaic virus 5' leader sequence (Gallie et al., Nucl. Acids Res. 15 (1987), 8693-8711).

Adaptors or linkers can be attached to the fragments to connect the DNA fragments with each other. Preferably, the promoter and the terminator regions can be provided, in the direction of transcription, with a linker or polylinker comprising one or more restriction sites for insertion of this sequence. As a rule, the linker has 1 to 10, preferably 1 to 8, especially preferably 2 to 6, restriction sites. In general, the linker has a size of less than 100 bp within the regulatory regions, frequently less than 60 bp, but at least 5 bp. The promoter can be native, or homologous, or else foreign, or heterologous, relative to the host plant.

The invention furthermore relates to a gene structure comprising an ATP/ADP translocator gene and regulatory sequences linked operably to this gene and a vector

comprising an ATP/ADP translocator gene or a gene structure as described above. In this context, the vector may comprise additional regulatory nucleotide sequences, preferably from the group of the promoters, terminators or translation enhancers, and nucleotide sequences for the replication in a suitable host cell or for integration into its genome.

Using recombination and cloning techniques known per se, the gene structures can be cloned into suitable vectors which make possible their amplification in host cells such as, for example, plants, plant tissues or plant cells. Suitable vectors are described, inter alia, in "Methods in Plant Molecular Biology and Biotechnology" (CRC Press), Chapter 6/7, pp. 71-119 (1993).

Suitable as cloning vectors for *E. coli* as host cell are, in particular, pBR332, pUC series, M13mp series and pACYC1 84. Especially preferred are binary vectors which are capable of replication both in *E. coli* and in, for example, agrobacteria. An example of a binary vector which may be mentioned is pBIN19 (Bevan et al., Nucl. Acids Res. 12 (1984), 8711). For example, the gene structure according to the invention may also be incorporated into tobacco transformation vector pBIN-AR-TP.

The present invention furthermore relates to a method of generating an above-described transformed plant, wherein an ATP/ADP translocator gene, a gene structure or a vector of the above-described type is transferred into the plant or tissue or cells thereof by recombinant methods. In general, the transfer of DNA is to be understood as meaning the transformation of plants, plant tissue or plant cells.

Suitable methods for the transformation and regeneration of plants from plant tissues or plant cells for

transient or stable transformation are protoplast transformation by polyethylene glycol-induced DNA uptake, the biolistic method with the gene gun - what is known as the particle bombardment method -, electroporation, the incubation of dry embryos in DNA-containing solution, microinjection and the agrobacterium-mediated gene transfer. The methods mentioned are described, for example, in B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press (1993), 128143 and in Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991), 205225).

The present invention thus makes possible the generation of economically valuable useful plants which are distinguished by a substantially increased amino acid content, in particular a substantially increased essential amino acid content.

The present invention furthermore relates to the use of the transformed plant as useful plant or fodder plant. Since the content of several essential amino acids may be increased in the useful plants according to the invention, in particular simultaneously, expensive supplementation of the feedstuffs with amino acids which previously had to be produced or obtained separately by conventional methods and externally admixed to the feed can advantageously be dispensed with.

The transformed plant in accordance with the invention, its seed and its progeny, and tissue or cells thereof or extracts thereof, can furthermore be used in sectors of agriculture, the feedstuff industry, the pharmaceutical industry or in the health sector.

In the following text, the present invention is illustrated in greater detail by use examples which,

however, do not limit the scope of the invention:

1. General cloning methods

Cloning methods such as, for example, restriction cleavages, agarose gel electrophoresis, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linking DNA fragments, transformation of E. coli cells, bacterial cultures, phage multiplication, and sequence analysis of recombinant DNA, were carried out as described by Sambrook et al. (1989, Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6).

The bacterial strains used (E. coli, XL-I Blue) were obtained from Stratagene (Heidelberg) or Qiagen (Hilden). The agrobacterial strain used for the transformation of the plants (Agrobacterium tumefaciens, C58C1 with the plasmid pGV2260 or pGV3850kan) was described by Deblaere et al. in Nucl. Acids Res. 13 (1985), 4777. As an alternative, the agrobacterium strain LBA4404 (Clontech) or other suitable strains may also be employed.

The vectors pUC19 (Yanish-Perron, Gene 33 (1985), 103-119) pBluescript SK- (Stratagene), pGEM-T (Promega), pZerO (Invitrogen) pBin19 (Bevan et al., Nucl. Acids Res. 12 (1984), 8711-8720) and pBinAR (Höfgen and Willmitzer, Plant Science 66 (1990), 221-230) may be used for cloning.

2. Transformation of agrobacteria

Agrobacterium tumefaciens was transformed following the method of Höfgen and Willmitzer (Nucl. Acid Res. (1988) 16, 9877). The agrobacteria were grown in YEB medium (Vervliet et al., J. Gen. Virol. (1975) 26, 33).

3. Sequence analysis of recombinant DNA

Recombinant DNA molecules were sequenced using a Licor laser fluorescence DNA sequencer (sold by MWG Biotech.,

Ebersbach) following the method of Sanger (Sanger et al., Proc. Natl. Acad. Sci. USA 74 (1977), 5463-5467).

4. Construction of a plant transformation vector with AATP1 in sense orientation

To construct a vector for transforming plants, a 2230 bp EcoRV/BamHI fragment of the Arabidopsis thaliana AATP1 cDNA (the cloning of AATP1 from Arabidopsis thaliana is described in Kampfenkel et al., FEBS Letters 374 (1995), 351-355 and Neuhaus et al., The Plant Journal 11: 73-82) is ligated into an SmaI/EcoRV- and BamHI-cut vector pBinAR (Höfgen and Willmitzer, Plant Science 66 (1990), 223-230). Insertion of the cDNA fragment gives rise to a gene construct comprising the cauliflower mosaic virus 35S promoter (540 bp) and the protein-encoding region of the Arabidopsis thaliana ADP/ATP translocator 1 (AATP1). The cDNA fragment is fused in sense orientation to the 35S promoter in pBinAR. The polyadenylation signal of the Agrobacterium tumefaciens octopine synthase gene (215 bp) follows in the 3' direction of the inserted AATP1 fragment.
The overall size of the plasmid pBIN AR-AATP1 (Fig. 3) is approx. 14.2 kb.

5. Insertion of the plasmid pBINAR-ATTP1 into the genome of potato plants

The plasmid is transferred into potato plants with the aid of Agrobacterium tumefaciens as described by Rocha-Sosa et al. (EMBO J. 8 (1989), 23-29). Transgenic potato plants with an increased level of the plastid ADP/ATP translocator 1 mRNA acted as positive control for the transformation. Detection is by Northern blot analysis. To this end, RNA is isolated from potato leaf and tuber tissue following standard protocols. 50 µg of RNA are separated on an agarose gel (1.5% agarose, 1 × MEN buffer, 16.6% formaldehyde). Following electrophoresis, the RNA is transferred to a Hybond N nylon membrane (Amersham, UK) by capillary blotting, using

20 × SSC. The RNA is immobilized on the membrane by UV irradiation, and the membrane is prehybridized for 2 hours in phosphate hybridization buffer (Sambook et al., 1989, Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6) and subsequently hybridized for 10 hours by adding the radiolabelled probe.

6. Construction of a plant transformation vector with AATP1 in antisense orientation

To construct a vector for the transformation of plants, a 1 265 bp BamHI/NdeI fragment in which the NdeI cleavage site is made blunt-ended with T4 polymerase is ligated from the coding region of the S. tuberosum AATP1 cDNA (description of the potato AATP1 cloning in Tjaden et al., 1998, The Plant Journal 16: 531-540) into an SmaI- and BamHI-cut vector pBinAR (Höfgen and Willmitzer, Plant Science 66 (1990), 221-230). The NdeI cleavage site is located in the AATP1 cDNA, and the BamHI cleavage site is derived from vector pTM1 (Tjaden et al., 1998, The Plant Journal 16: 531-540). Insertion of the cDNA fragment gives rise to a gene construct comprising the cauliflower mosaic virus 35S promoter (540 bp) and a 1 265 bp region of an ADP/ATP translocator 1 from S. tuberosum (AATP1 S.t.) in antisense orientation. The fragment was fused with the 35S promoter in pBinAR. The polyadenylation signal of the Agrobacterium tumefaciens octopine synthase gene (215 bp) follows in the 3' direction of the inserted AATP1 fragment.

The overall size of the plasmid pBIN AR-AS-AATP1 (Fig. 4) is approx. 13.3 kb.

7. Introduction of the plasmid pBINAR-ASAATP1 into the genome of potato plants

The plasmid is transferred similarly to the procedure described under item 5.

As the result of the transformation, transgenic potato plants showed a reduced level of the mRNA of a plastid

ADP/ATP translocator. This is detected by Northern blot analysis. To this end, RNA is isolated from potato leaf and tuber tissue following standard protocols. 50 µg of RNA were separated on an agarose gel (1.5% agarose, 1 × MEN buffer, 16.6% formaldehyde). Following electrophoresis, the RNA was transferred to a Hybond N nylon membrane (Amersham, UK) by capillary blotting, using 20 × SSC. The RNA is immobilized on the membrane by UV irradiation. The membrane is prehybridized for 2 hours in phosphate hybridization buffer (Sambrook et al., loc. cit.) and subsequently hybridized for 10 hours by adding the radiolabelled probe.

8. Amino acid analysis

The amine acids (with the exception of proline) were measured in ethanolic extracts following HPLC separation (method of Geigenberger et al., 1996, Plant Cell & Environ. 19: 43-55).

8.1 Preparation of the ethanolic extract

In each case two potato discs (total fresh weight approx. 0.2 g) are extracted for 30 minutes at 80°C in two successive steps using in each case 7 ml of 80% (v/v) ethanol and 7 ml of 50% ethanol. The total extract (approximate volume 14 ml) is used for the amino acid analysis.

8.2 Determination of the amino acid contents by HPLC

The amino acids were detected fluorometrically following pre-column derivatization of the primary amino group with o-phthaldialdehyde (OPA). To this end, an injector (Autosampler 465, Kontron, Eching) injects 35 µl of OPA reagent composed of a mixture of 5% (w/v) OPA in methanol, 0.8 M borate buffer (pH 10.4 with KOH) and 3-mercaptopropionic acid (10:90:1, v:v:v) at 4°C into 35 µl of extract. After 108 seconds, 20 µl of the derivatized sample were injected.

Mobile phase A is a mixture of 1 000 ml of 12 mM sodium

phosphate (pH 6.8) and 1.6 ml of tetrahydrofuran. Mobile phase B is composed of a mixture of 250 ml of 12 mM sodium phosphate (pH 6.8), 175 ml of methanol and 110 ml of acetonitrile. The separation conditions are as follows: minute 0-2, isocratic phase with 0% B, minute 2-11, linear gradient from 0 to 10% B, minute 11-17, 10% B, minute 17-27, linear gradient from 10 to 50% B, minute 27-38, linear gradient from 50 to 60% B, minute 38-44, linear gradient from 60 to 100% B, minute 44-46, 100% B, minute 46-48, 100% to 0% B, minute 48-60, 0% B. A Hypersil ODS column (particle size 3 µm, length 150 mm, diameter 4.6 mm, Knauer GmbH, Berlin) is used for the separation. The signals detected by the fluorimeter (SFM25, Kontron, Eching) (excitation wavelength = 330 nm, emission wavelength = 450 nm) are integrated and evaluated by the data processing system 450-MT (Kontron, Eching).

8.3 Determination of the proline content

The proline content is determined by the method of Bates et al., 1973, Plant Soil 39: 205-207.
500 µl of a mixture of 2 parts of 6 M H_3PO_4 and 3 parts of 75% acetic acid and 500 µl of ninhydrin solution (600 mg per 20 ml of 75% acetic acid) are added to 200 µl of extract. After incubation for 45 minutes at 95-100°C, the test mixture is placed on ice and mixed with 300 µl of toluene. Following phase separation, the top phase is transferred into a microcuvette, and the OD is measured at 515 nm. The proline content is determined by comparison with a calibrating plot (1-50 µM proline).

Tab. 1: Overview of the amino acid content in the Solanum tuberosum wild type and in the transformed potato plants comprising the ATP/ADP translocator gene in sense orientation (Sense-98 and Sense-62) or in antisense orientation (Antis-594 and Antis-595).

Genotype	Aspartate	Glutamate	**Aspartic acid**	Serine	Glutamine
Wild type	2.020	2.090	11.190	1.066	5.586
Sense-98	1.656	2.238	8.986	1.008	7.409
Sense-62	1.924	1.540	12.533	0.838	6.949
Antis-594	0.746	4.123	1.256	0.875	5.633
Antis-595	0.880	4.670	4.344	1.057	6.931
Genotype	Tyrosine	Valine	**Methionine**	Tryptophan	Phenyl-alanine
Wild type	1.201	3.589	0.986	0.519	1.544
Sense-98	1.840	4.010	1.098	0.780	2.286
Sense-62	1.440	3.633	0.920	0.506	1.620
Antis-594	0.474	2.620	0.403	0.143	2.039
Antis-595	0.228	2.340	0.510	0.019	1.716
Genotype	Glycine	**Threonine**	Histidine	Alanine	Arginine
Wild type	0.473	1.168	0.699	1.036	1.809
Sense-98	0.507	1.318	0.865	1.694	2.122
Sense-62	0.442	1.197	0.838	1.165	2.008
Antis-594	0.448	0.612	0.265	1.824	0.493
Antis-595	0.641	0.574	0.292	1.562	0.396
Genotype	**Isoleucine**	Leucine	**Lysine**	Proline	Free ASs, total
Wild type	1.450	0.212	1.027	0.595	41.7
Sense-98	1.819	0.296	1.310	0.552	44.7
Sense-62	1.445	0.195	1.291	0.546	43.9
Antis-594	0.681	0.142	0.270	0.451	27.7
Antis-595	0.535	0.124	0.228	0.470	33.0

All data in $\mu mol/gFW^{-1}$

Patent Claims:

1. Transformed plant and its progeny, characterized in that its regulatory sequences and/or gene copy number of an ATP/ADP translator gene are modified in such a way that it exhibits one or more amino acids simultaneously in modified amounts in comparison with a corresponding untransformed plant.

2. Transformed plant and its progeny according to Claim 1, characterized in that it exhibits an increased transport capacity for ATP into the chloroplast membrane.

3. Transformed plant and its progeny according to Claim 1 or 2, characterized in that it exhibits predominantly one or more essential amino acid(s) in modified amounts.

4. Transformed plant and its progeny according to one of Claims 1 to 3, characterized in that it exhibits one or more essential amino acid(s) whose content is increased over that of the untransformed plant.

5. Transformed plant and its progeny according to one of Claims 1 to 4, characterized in that it is a useful plant.

6. ATP/ADP translocator gene for use in a plant according to one of Claims 1 to 5 with an Arabidopsis thaliana nucleotide sequence(EMBL Accession No. Z49227) encoding the amino acid sequence shown in Fig. 1.

7. ATP/ADP translocator gene according to Claim 6 with a naturally, chemically synthesized, modi-

fied, artificially generated nucleotide sequence with essentially the same action or with heterologous nucleotide sequences encoding an ATP/ADP translocator or allelic variations or isoforms thereof or with mixtures thereof.

8. ATP/ADP translocator gene according to Claim 6 or 7 with operably linked regulatory nucleotide sequences.

9. ATP/ADP translocator gene according to one of Claims 6 to 8 with an upstream, operably linked promoter.

10. Gene structure comprising an ATP/ADP translocator gene according to one of Claims 6 to 9 and regulatory sequences linked operably to this gene.

11. Vector comprising an ATP/ADP translocator gene according to one of Claims 6 to 9 or a gene structure according to Claim 10.

12. Vector according to Claim 11 comprising additional regulatory nucleotide sequences, preferably from the group of the promoters, terminators or translation enhancers, and nucleotide sequences for the replication in a suitable host cell or for integration into its genome.

13. Seeds of the plant according to one of Claims 1 to 5.

14. Tissue or cells or material capable of propagation from the plant according to one of Claims 1 to 5.

15. Method of generating a plant with an increased amino acid content according to one of Claims 1 to 5, characterized in that an ATP/ADP translocator gene according to one of Claims 6 to 9 or a gene

structure according to Claim 10 or a vector according to Claim 11 or 12 is transferred by recombinant methods.

16. Use of the transformed plant according to one of Claims 1 to 5 as useful plant or fodder plant.

17. Use of the transformed plants according to one of Claims 1 to 5 or of tissue or cells thereof or of extracts thereof in sectors of agriculture, the feedstuff industry, the pharmaceutical industry or in the health sector.

(12) NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT) VERÖFFENTLICHTE INTERNATIONALE ANMELDUNG

(19) Weltorganisation für geistiges Eigentum
Internationales Büro

(43) Internationales Veröffentlichungsdatum
22. März 2001 (22.03.2001)

PCT

(10) Internationale Veröffentlichungsnummer
WO 01/20009 A1

(51) Internationale Patentklassifikation[7]: C12N 15/82, 15/29, C07K 14/415, A01H 5/00

(21) Internationales Aktenzeichen: PCT/EP00/07625

(22) Internationales Anmeldedatum:
5. August 2000 (05.08.2000)

(25) Einreichungssprache: Deutsch

(26) Veröffentlichungssprache: Deutsch

(30) Angaben zur Priorität:
199 44 212.6 15. September 1999 (15.09.1999) DE

(71) Anmelder *(für alle Bestimmungsstaaten mit Ausnahme von US)*: **BASF AKTIENGESELLSCHAFT** [DE/DE]; D-67056 Ludwigshafen (DE).

(72) Erfinder; und

(75) Erfinder/Anmelder *(nur für US)*: **REINDL, Andreas** [DE/DE]; Albertine-Scherer-Str. 21, D-67134 Birkenheide (DE). **GEIGENBERGER, Peter, Ludwig** [DE/DE]; Frauenpfad 31, D-69221 Dossenheim (DE). **NEUHAUS, Horst-Ekkerhard** [DE/DE]; Sachsenweg 29, D-49086 Osnabrück (DE). **GRAEVE-KAMPFENKEL, Karlheinz** [DE/DE]; Im Sampel 10a, D-55246 Mainz-Kostheim (DE). **MÖHLMANN, Torsten** [DE/DE]; Gelerstr. 17, D-32257 Bünde (DE). **TJADEN, Joachim** [DE/DE]; Kreuzweg 9, D-49134 Lechtingen-Wallenhorst (DE).

(74) Anwalt: **FITZNER, Uwe**; Lintorfer Strasse 10, D-40878 Ratingen (DE).

(81) Bestimmungsstaaten *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Bestimmungsstaaten *(regional)*: ARIPO-Patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), eurasisches Patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europäisches Patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI-Patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Veröffentlicht:
— *Mit internationalem Recherchenbericht.*

Zur Erklärung der Zweibuchstaben-Codes, und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

(54) **Title:** PLANTS HAVING ALTERED AMINO ACID CONTENTS AND METHOD FOR THE PRODUCTION THEREOF

(54) **Bezeichnung:** PFLANZEN MIT VERÄNDERTEM AMINOSÄUREGEHALT UND VERFAHREN ZU DEREN HERSTELLUNG

(57) **Abstract:** The invention relates to transformed plants and their descendants, which have altered regulative sequences and/or number of gene copies of the ATP/ADP-translocator-gene in such a manner that they have one or more amino acids simultaneously in altered amounts in comparison with a non-transformed plants. The invention also relates to a method for the production of said plants in addition to their use as crop plants or in the animal feedstuffs industry.

(57) **Zusammenfassung:** Die vorliegende Erfindung betrifft transformierte Pflanzen und deren Nachkommen, die in den regulativen Sequenzen und/oder der Genkopienzahl des ATP/ADP-Translokator-Gens derart verändert sind, dass sie gegenüber einer nicht transformierten Pflanze eine bis mehrere Aminosäuren gleichzeitig in veränderten Mengen aufweisen. Ferner betrifft die vorliegende Erfindung ein Verfahren zur Herstellung dieser Pflanzen sowie deren Verwendung als Nutzpflanze oder in Bereichen der Futtermittelindustrie.

WO 01/20009 A1

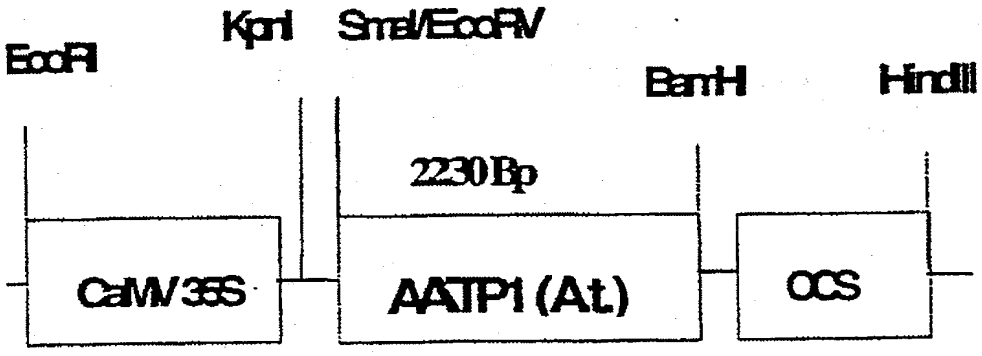
Fig. 1: Arabidopsis thaliana cDNA corresponding to the coding region of the chloroplast ATP/ADP translocator 1 (EMBL Accession Number Z49227)

atggaagctgtgattcaaaccagagggcttctctctttacccaccaaacccatcggagtgagaagcca
acttcagccttcccatggcttaaagcagagacttttcgccgcgaagccaagaaatctacatgggtgtct
ctatcctttaacgggcacaagaaatttcaaacctttgagccaaccctgcatgggatttcgatttcccaca
aagagagaagcaccgagttcatatgcaaggcggaggcgcggctgctggcgacggagctgtcttcg
gcgaagcgattccgcagctgttgtagcctcgcggaagattttcggtgtggaggttgcaaccttgaaaaa
gattatccctttaggattgatgttcttttgtattcttttcaattacacaattctgagggatacaaaggatgtcttg
gtggtgacggcgaaaggaagttctgctgagattataccttttcttgaagacttgggtgaatcttcctatggc
cattgggtttatgctcctctacactaaactctccaatgttctctccaagaaggctctgttttacactgttattgtc
cctttcatcatctactttgggggctttggtttcgtcatgtaccctctcagcaactatattcacccggaagctct
cgcagataagctccttacaaccctcggcccaagattcatgggtcctattgcaatattgcggatttggagtt
tctgtttgttttatgttatggctgagctttggggtagtgtggtggtctcagttctcttctggggctttgctaatcag
atcacaactgtggatgaagccaagaaattctatcctttgttcggcattggagccaatgttgcactgattttc
tcaggaagaaccgtgaaatacttctctaacttgagaaagaatcttggtcctggagttgacggcagtttcg
ttgaaagccatgatgagcattgtggtgggaatgggactcgcatttgtctctctattggtgggtcgaataga
tatgttcctcttccaacccgtagcaagaacaagaaggagaaaccgaagatgggaacgatggaaag
cttgaagttcttggtatcatcaccatacattagagatcttgctactttagtggtggcatacggtattagtatca
atcttgtggaagtcacatggaaatcaaagcttaaagctcagttccctagcccgaatgagtactcagcatt
tatgggagcattctcaacctgcacgggtgttgcaacattcacaatgatgcttctcagccaatacgtattca
ataagtatggttggggagtagctgcaaagatcaccccaactgttctgctattgactggtgttgcgttcttct
ctctaatattgtttggcggcccattcgcaccacttgttgccaagcttggtatgacaccgctacttgcagctgt
gtatgtcggtgcccttcagaatatcttcagcaagagtgccaagtacagcttgttcgacccttgcaaagaa
atggcctatatcccattggatgaggacaccaaggttaaaggcaaagctgcgattgacgtggtctgcaa
cccattaggaaaatcaggggagctttaatacagcagttcatgatcttatcctttggatcactagcgaatt
caacgccgtatctaggaatgatcttgttggttattgtcactgcgtggttagctgcagctaagtcgctggag
ggacagttcaacagcttgcgtctgaagaagagcttgagaaggaaatggagagagcttcatcggtga

Fig. 2: Solanum tuberosum cDNA corresponding to the coding region of the chloroplast ATP/ADP translocator 1 (EMBL Accession Number Y10821)

atggaaggtgttttacaaacaagagggcttctttctttgccttctaaacccaaaatcaaggctttttacccat
tgcctcaaggggtctaaggaacagattcaattctttaagtagtttaaagcctaatcctcttaatggggttt
ctttatcttcaaatgggtttcaaaaagttcaaggctttgacacaaagcctcagttgtttggccaaaagaag
aggtgttttccaatatgcaaagctgaggctgctgctgctgctggtgcagctgatggacagccacttttgtt
gaaaaggagcaacctaagtttatggggattgaacttgtgacccttaagaaaattataccacttggggcg
atgttcttttgtattctgtttaattatacaatccttagggatactaaggatgtgttggttgtaacagctaaaggg
tccagtgctgagattatccctttcttgaaaacttgggtgaatttgcctatggctattggattcatgcttttgtac
acaaagttggctaatgtgttgtcaaaggaggctcttttttatactgttatacttccttttattgcattctttggggc
gtttggttttgttttgtatcctcttagcaattactttcaccctacagcttttgctgataagcttctcaataccttgg
tccaagatttcttggaccaattgctattctgaggatctggagtttctgcttgttctatgtcatggctgagctttg
gggaagtgtggtggtttcagtactcttttggggatttgctaatcagatcacgactgtcgatgaggctaaga
gattctatcctttgtttggacttggagcgaatgttgctcttattttctctggtcgcacagtgaagtacttttctag
cttgagaagctctttaggtcctggagttgatggttgggctatctccctgaaaggaatgatgagtattgttgt
gatgatgggtggggcaatctgtttcttttactggtgggtgaatagaaatgttgctctcccaactcgtagcaa
gaagaagaaggtaaaacctaacatgaccacaatggagagcttgaagttcttggtctcttcaaaatatat
cagggatcttgccacattggttgtagcatatggcattagtatcaaccttgttgaagttacatggaagtcaa
agctcaaagctcagttcccaagccccaatgaatactcctcattcatgggtgacttctcaactgctactgg
aatagcaactttcacaatgatgttgttaagtcaatggattttcgacaagtatgggtggggagcagcagcc
aagataacacctacagtcttgctccttaccggagttggtttcttctccctgcttttgtttggggcacctctagc
acctactcttgcgaagtttggaatgactcctcttctagcagctgtctatgtgggtgcaatgcagaacattttc
agtaagagtgcaaagtatagtttgtttgacccctgcaaagaaatggcctacattcctttggatgaggaca
ccaaggttaaagggaaggcagcaatcgatgttgtctgcaatccactgggaaagtctggaggagctttg
atacaacagttcatgattttgacttttggttcacttgccagctcgacaccctaccttggcggtgtgctcttagt
aattgttcttgcatggttgggagcagccaagtctttggatggacagttcactcaattacgccaagaagaa
gatcttgagaaggaaatggagagagcatcgttgaagatccctgtcgtgtctcaaaatgaaaatggaa
atggtcctctctcaagtgagtcatcactaaatcccgctggaggtgactctaccaacgcttcatcggaacc
ctcctccccaaggagcctgtaa

Fig. 3: Plant transformation vector pBIN-AR-AATP1 for expressing the ATP/ADP translocator in sense orientation



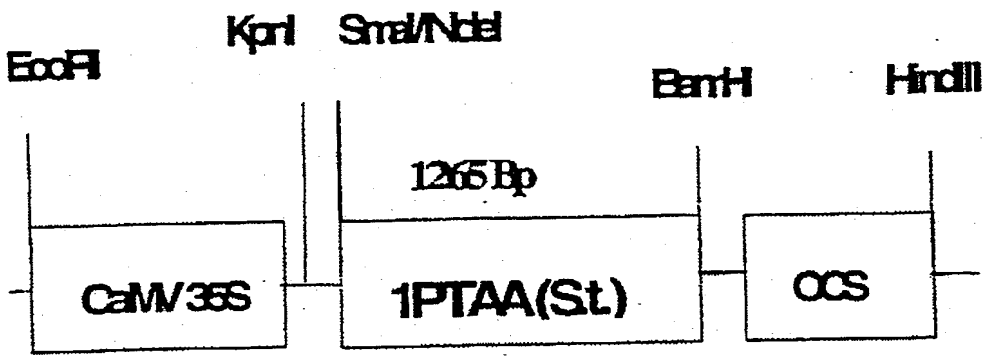
CaMV 35S: cauliflower mosaic virus 35S promoter

AATP1 (A.t.): EcoRV/BamHI fragment of the Arabidopsis thaliana ATP/ADP translocator 1 in sense orientation

OCS: polyadenylation signal of the Agrobacterium tumefaciens octopine synthase gene

Those restriction sites which cut the vector only once are also shown.

Fig. 4: Plant transformation vector pBIN-AR-AATP1-AS for expressing the ATP/ADP translocator in antisense orientation



CaMV 35S: cauliflower mosaic virus 35S promoter

1PTAA (S.t.): BamHI/NdeI fragment of the Solanum tuberosum ATP/ADP translocator gene in antisense orientation

OCS: polyadenylation signal of the Agrobacterium tumefaciens octopine synthase gene

Those restriction sites which cut the vector only once are also shown.

Declaration, Power of Attorney and Petition

We (I), the undersigned inventor(s), hereby declare(s) that:

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

the specification of which

[ ] is attached hereto.

[ ] was filed on _____ as

Application Serial No. _____

and amended on _____ .

[x] was filed as PCT international application

Number *PCT/EP/00/07625*

on *05 August 2000* ,

and was amended under PCT Article 19

on _____ (if applicable).

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s)

Application No.	**Country**	**Day/Month/Year**	**Priority Claimed**	
19944212.6	*Germany*	*15 September 1999*	[x] Yes	[ ] No

pdusnpct004 - 518

We (I) hereby claim the benefit under Title 35, United States Codes, § 119(e) of any United States provisional application(s) listed below.

(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

We (I) hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	**Filing Date**	**Status (pending, patented, abandoned)**

And we (I) hereby appoint **Nixon & Vanderhye P.C.**, Attorneys at Law, 1100 North Glebe Road, Arlington, Virginia 22201-4714, our attorneys, with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to sign the drawings, to receive the patent, and to transact all business in the Patent Office connected therewith.

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

1-00

Andreas Reindl
NAME OF INVENTOR

Signature of Inventor

Date

Albertine-Scherer-Str.21
67134 Birkenheide
Germany DE
Citizen of: Germany
Post Office Address: same as residence

2-00

Peter Ludwig Geigenberger
NAME OF INVENTOR

Signature of Inventor

Date

Frauenpfad 31
69221 Dossenheim
Germany DE
Citizen of: Germany
Post Office Address: same as residence

3-00

Horst-Ekkerhard Neuhaus
NAME OF INVENTOR

Signature of Inventor

Date

Sachsenweg 29
49086 Osnabrück
Germany DE
Citizen of: Germany
Post Office Address: same as residence

4-00

Karlheinz Graeve-Kampfenkel
NAME OF INVENTOR

Signature of Inventor

Date

Im Sampel 10a
55246 Mainz-Kostheim
Germany DE
Citizen of: Germany
Post Office Address: same as residence

5-00

Torsten Möhlmann
NAME OF INVENTOR

Torsten Möhlmann
Signature of Inventor

Date

Gelertstr.17
32257 Bünde
Germany DE X
Citizen of: Germany
Post Office Address: same as residence

6-00

Joachim Tjaden
NAME OF INVENTOR

Joachim Tjaden
Signature of Inventor

Date

Kreuzweg 9
49134 Lechtingen–Wallenhorst
Germany DE X
Citizen of: Germany
Post Office Address: same as residence

SEQUENCE LISTING

<110> BASF AG

<120> PLANTS WITH A MODIFIED AMINO ACID CONTENT AND THEIR GENERATION

<130> 3557-12
<140> 199 44 212.6
<141> 1999-09-14

<160> 2

<170> PatentIn Ver. 2.1

<210> 1
<211> 1770
<212> DNA
<213> Arabidopsis thaliana

<400> 1
atggaagctg tgattcaaac cagagggctt ctctctttac ccaccaaacc catcggagtg 60
agaagccaac ttcagccttc ccatggctta aagcagagac ttttcgccgc gaagccaaga 120
aatctacatg ggtgtctcta tcctttaacg ggcacaagaa atttcaaacc tttgagccaa 180
ccctgcatgg gatttcgatt tcccacaaag agagaagcac cgagttcata tgcaaggcgg 240
aggcgcggct gctggcgacg gagctgtctt cggcgaagcg attccgcagc tgttgtagcc 300
tcgcggaaga ttttcggtgt ggaggttgca accttgaaaa agattatccc tttaggattg 360
atgttctttt gtattctttt caattacaca attctgaggg atacaaagga tgtcttggtg 420
gtgacggcga aaggaagttc tgctgagatt ataccttttct tgaagacttg ggtgaatctt 480
cctatggcca ttgggtttat gctcctctac actaaactct ccaatgttct ctccaagaag 540
gctctgtttt acactgttat tgtccctttc atcatctact ttgggggctt tggtttcgtc 600
atgtaccctc tcagcaacta tattcacccg gaagctctcg cagataagct ccttacaacc 660
ctcggcccaa gattcatggg tcctattgca atattgcgga tttggagttt ctgtttgttt 720
tatgttatgg ctgagctttg gggtagtgtg gtggtctcag ttctcttctg gggctttgct 780
aatcagatca caactgtgga tgaagccaag aaattctatc ctttgttcgg cattggagcc 840
aatgttgcac tgatttttctc aggaagaacc gtgaaatact tctctaactt gagaaagaat 900
cttggtcctg gagttgacgg cagtttcgtt gaaagccatg atgagcattg tggtgggaat 960
gggactcgca tttgtctctc tattggtggg tcgaatagat atgttcctct tccaacccgt 1020
agcaagaaca agaaggagaa accgaagatg ggaacgatgg aaagcttgaa gttcttggta 1080
tcatcaccat acattagaga tcttgctact ttagtggtgg catacggtat tagtatcaat 1140
cttgtggaag tcacatggaa atcaaagctt aaagctcagt tccctagccc gaatgagtac 1200
tcagcattta tgggagcatt ctcaacctgc acgggtgttg caacattcac aatgatgctt 1260
ctcagccaat acgtattcaa taagtatggt tggggagtag ctgcaaagat caccccaact 1320
gttctgctat tgactggtgt tgcgttcttc tctctaatat tgtttggcgg cccattcgca 1380
ccacttgttg ccaagcttgg tatgacaccg ctacttgcag ctgtgtatgt cggtgccctt 1440
cagaatatct tcagcaagag tgccaagtac agcttgttcg acccttgcaa agaaatggcc 1500
tatatcccat tggatgagga caccaaggtt aaaggcaaag ctgcgattga cgtggtctgc 1560
aacccattag gaaaatcagg gggagcttta atacagcagt tcatgatctt atcctttgga 1620
tcactagcga attcaacgcc gtatctagga atgatcttgt tggttattgt cactgcgtgg 1680
ttagctgcag ctaagtcgct ggagggacag ttcaacagct tgcgtctgaa gaagagcttg 1740
agaaggaaat ggagagagct tcatcggtga 1770

<210> 2
<211> 1896
<212> DNA
<213> Solanum tuberosum

<400> 2

atggaaggtg ttttacaaac aagagggctt ctttctttgc cttctaaacc caaaatcaag 60
gctttttacc cattgcctca agggggtcta aggaacagat tcaattcttt aagtagttta 120
aagcctaatc ctcttaatgg ggtttcttta tcttcaaatg ggtttcaaaa agttcaaggc 180
tttgacacaa agcctcagtt gtttggccaa aagaagaggt gttttccaat atgcaaagct 240
gaggctgctg ctgctgctgg tgcagctgat ggacagccac tttttgttga aaaggagcaa 300
cctaagttta tggggattga acttgtgacc cttaagaaaa ttataccact tggggcgatg 360
ttcttttgta ttctgtttaa ttatacaatc cttagggata ctaaggatgt gttggttgta 420
acagctaaag ggtccagtgc tgagattatc cctttcttga aaacttgggt gaatttgcct 480
atggctattg gattcatgct tttgtacaca aagttggcta atgtgttgtc aaaggaggct 540
ctttttata ctgttatact tccttttatt gcattctttg gggcgtttgg ttttgttttg 600
tatcctctta gcaattactt tcaccctaca gcttttgctg ataagcttct caataccctt 660
ggtccaagat ttcttggacc aattgctatt ctgaggatct ggagtttctg cttgttctat 720
gtcatggctg agctttgggg aagtgtggtg gtttcagtac tcttttgggg atttgctaat 780
cagatcacga ctgtcgatga ggctaagaga ttctatcctt tgtttggact tggagcgaat 840
gttgctctta ttttctctgg tcgcacagtg aagtactttt ctagcttgag aagctcttta 900
ggtcctggag ttgatggttg ggctatctcc ctgaaaggaa tgatgagtat tgttgtgatg 960
atgggtgggg caatctgttt cttttactgg tgggtgaata gaaatgttgc tctcccaact 1020
cgtagcaaga agaagaaggt aaaacctaac atgaccacaa tggagagctt gaagttcttg 1080
gtctcttcaa aatatatcag ggatcttgcc acattggttg tagcatatgg cattagtatc 1140
aaccttgttg aagttacatg gaagtcaaag ctcaaagctc agttcccaag ccccaatgaa 1200
tactcctcat tcatgggtga cttctcaact gctactggaa tagcaacttt cacaatgatg 1260
ttgttaagtc aatggatttt cgacaagtat gggtggggag cagcagccaa gataacacct 1320
acagtcttgc tccttaccgg agttggtttc ttctccctgc ttttgtttgg ggcacctcta 1380
gcacctactc ttgcgaagtt tggaatgact cctcttctag cagctgtcta tgtgggtgca 1440
atgcagaaca ttttcagtaa gagtgcaaag tatagtttgt ttgacccctg caaagaaatg 1500
gcctacattc ctttggatga ggacaccaag gttaaaggga aggcagcaat cgatgttgtc 1560
tgcaatccac tgggaaagtc tggaggagct ttgatacaac agttcatgat tttgacttt 1620
ggttcacttg ccagctcgac accctacctt ggcggtgtgc tcttagtaat tgttcttgca 1680
tggttgggag cagccaagtc tttggatgga cagttcactc aattacgcca agaagaagat 1740
cttgagaagg aaatggagag agcatcgttg aagatccctg tcgtgtctca aaatgaaaat 1800
ggaaatggtc ctctctcaag tgagtcatca ctaaatcccg ctggaggtga ctctaccaac 1860
gcttcatcgg aaccctcctc cccaaggagc ctgtaa 1896